Coexpression of Neurofilament and Keratin Proteins in Cutaneous Neuroendocrine Carcinoma Cells*

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Four cases of neuroendocrine carcinomas (NECA) of the skin were studied by indirect immunofluorescence, using a monoclonal antikeratin antibody and a polyclonal antineurofilament antibody. Fifty to ninety percent and 80 to >95% of the NECA cells stained with the antineurofilament antibody and the antikeratin antibody, respectively. Using double-labeling indirect immunofluorescence we could also demonstrate that, in 3 cases studied, some of the NECA cells, but not all, stained with both antikeratin and antineurofilament antibodies. These results, together with the recent knowledge of the intermediate filament protein type of normal Merkel cells (MC), tend to support the hypothesis that NECA cells do not originate from epithelial MC but from dermal neuroendocrine cells. A dual concept of intraepithelial MC and extraepithelial intradermal neuroendocrine cells, "from possible distinct origin," is proposed. Such a system has already been suggested for the neuroendocrine cells of the appendix and bronchial mucosae. *J Invest Dermatol 86:74–77, 1986*

The neuroendocrine carcinoma (NECA) of the skin was first described by Toker [1] under the designation of trabecular carcinoma of the skin, and later called Merkel cell carcinoma [2]. NECA usually occurs as a well-defined, reddish or brownish, painless nodule, 0.5–12.0 cm in size [2–6]. It is covered by thin, shiny skin, but may be ulcerated. It is preferentially located in the head and neck regions or on the extremities of elderly patients. The histologic features of NECA are characteristic [1–3,7,8]. The tumor cells have a uniform histologic appearance with round or oval vesicular nuclei and prominent nucleoli. The cells form irregular sheets, and often anastomosing cords or trabeculae that infiltrate the collagen fibers of the dermis, and the subcutaneous fat. Rosette-like structures may be observed. Mitoses are common. At the ultrastructural level, the tumor cells demonstrate numerous membrane-bound, dense-core granules ("neurosecretory granules") 120–220 nm in size, some cytoplasmic processes resembling microvilli, infrequent desmosome-like intercellular junctions, as well as cytoplasmic intermediate filaments [2,4–11]. Intranuclear rodlet-like inclusions may be observed [10]. Due to striking ultrastructural similarities to normal Merkel cells (MC), it has been proposed [2], and largely accepted [3–6,8–11] that NECA originate from MC. However, no definite histogenetic relationship between normal MC and NECA has ever been documented. Furthermore, if these tumors do in fact originate from MC, they must have their origin in the rarely described dermal MC [12–15], because intraepidermal or intraappendageal involvement by the neoplastic process is only occasionally observed [16,17].

In order to further elucidate the histogenesis of NECA cells the cytoskeleton was studied, because tumor cells continue to express the intermediate filament proteins of their cell of origin [18,19].

In the course of such a study, and simultaneously with the use of other differentiation-related specific cellular markers,‡ we observed that NECA express both cytokeratin and neurofilament proteins. Using a double-labeling indirect immunofluorescence (IIF) technique we now report that both intermediate filament proteins are present within the same cells [20], and discuss the implications of these findings for the histogenesis of NECA.

MATERIALS AND METHODS

Patients Specimens were obtained from 2 male and 2 female patients. The ages ranged from 49–83 years. Two lesions were located in the buttock, one in the groin, and one in the thigh. None of the patients had any demonstrable visceral neuroendocrine tumor, thus excluding the possibility of a cutaneous metastasis.

Specimens The specimens were obtained from biopsy or surgical excision material, under local anesthesia with 1% Xylocaine with epinephrine. They were immediately divided into 3 parts. One of them was processed for routine histology, another was fixed in 2% glutaraldehyde for electron microscopy, and the last one was frozen in Optimum Cutting Temperature (OCT) compound (Ames Co., Division of Miles Laboratories, Elkhard, Indiana) by suspension in isobutane cooled with dry ice. Control

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Abbreviations:
DIIF: double-labeling in direct immunofluorescence
FITC: fluorescein isothiocyanate
IIF: indirect immunofluorescence
MC: Merkel cell
NECA: neuroendocrine carcinoma
NHS: normal human skin
TRITC: tetramethylrhodamine isothiocyanate

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normal human skin (NHS) was obtained from plastic surgical excisions and frozen as described previously.

Antibodies The rabbit polyspecific polyclonal antineurofilament antibody was prepared in the laboratory of one of us (D.D.) and has been described in detail elsewhere [21]. The mouse polyspecific monoclonal antibody KL-1 was purchased from Immunotec (Marseille, France) and it has been recently described [22]. Fluorescein isothiocyanate (FITC)-labeled goat anti-mouse IgG antiserum and tetramethylrhodamine isothiocyanate (TRITC)-labeled goat anti-rabbit Ig antiserum were purchased from Cappell Laboratories (Cochranville, Pennsylvania).

IIF and Double-Labeling Indirect Immunofluorescence (DIIF) IIF and DIIF were performed according to standard techniques that have been previously described in detail elsewhere [23,24].

Controls IIF and DIIF controls included NHS and NECA specimens incubated with phosphate-buffered saline, rabbit preimmune serum, and mouse ascitic fluid as primary antibodies. Primary antibodies were also omitted to assess staining due only to FITC and TRITC antisera.

RESULTS

All four tumors presented the light and electron microscopic characteristic features of NECA. The tumor cells formed elongated anastomosing cords, irregular trabeculae, or large masses with a pushing border infiltrating between the collagen fibers of the dermis, and extending into the subcutaneous fat. In case no. 2, the tumor was found only in the subcutaneous fat. The overlying epidermis as well as the cutaneous adnexa were unremarkable and separated from the tumoral proliferation by a thin band of dermis. There were fibrovascular septa separating large tumoral cords, and a moderate chronic inflammatory infiltrate. The tumor cells were uniform with round or oval vesicular nuclei. Nucleoli were often prominent. The cytoplasmic membrane was ill defined, and there was scanty basophilic to lavender cytoplasm. Mitotic figures were usually numerous. In some instances, rosette-like circular structures, but not true acini, were observed. In frozen sections the epidermis was present only in case no. 1.

An electron microscopy study (data not shown) confirmed the neuroendocrine differentiation of the tumors. The cells appeared rather uniform with a round to oval nucleus, a well-developed rough endoplasmic reticulum, Golgi complexes, and varying numbers of mitochondria. Intermediate filaments (10 nm) were observed mainly in the perinuclear area, but paranuclear aggregates were also common. Dense-core, membrane-bound granules were noted in each case. Intercellular desmosome-like structures were also present.

NHS and NECA IIF controls always remained negative. Within the epidermis, the antikeratin antibody KL-1 stained the epidermis, but not the basal cell layer, as well as the sweat gland and duct epithelia. The antineurofilament antibody stained the axons of the cutaneous nerves.

When a single-labeling IIF was used, 50–90% of the carcinomatous cells stained with antineurofilament antibody (case nos. 1 and 3, 90%; case no. 2, 50%; case no. 4, 10%; Fig 1*a*). Within the cytoplasm the neurofilament immunoreactivity did not appear diffusely scattered, but was arranged in cords and balls mainly at the periphery of the cells. No specific relationship between the stained and unstained cells was observed. About the same proportion of cells (80 to >95%) stained also with the antikeratin antibody (case nos. 1 and 3, 80%; case no. 2, >95%; case no. 4, not done; Fig 1*b*). The staining pattern was similar to that of the neurofilaments, but also occasionally appeared diffuse.

This dual staining implicates an apparent overlap between the expression of neurofilament and keratin proteins, and implies that the same cell might express both intermediate filament proteins. To test this hypothesis a double-labeling technique was used. In the 3 cases studied with this technique we could observe that about 30–80% of the NECA cells did express both the keratin and neurofilament proteins (Fig 2*a*,*b*). The staining pattern was similar to that described previously with the antineurofilament and antikeratin antisera.

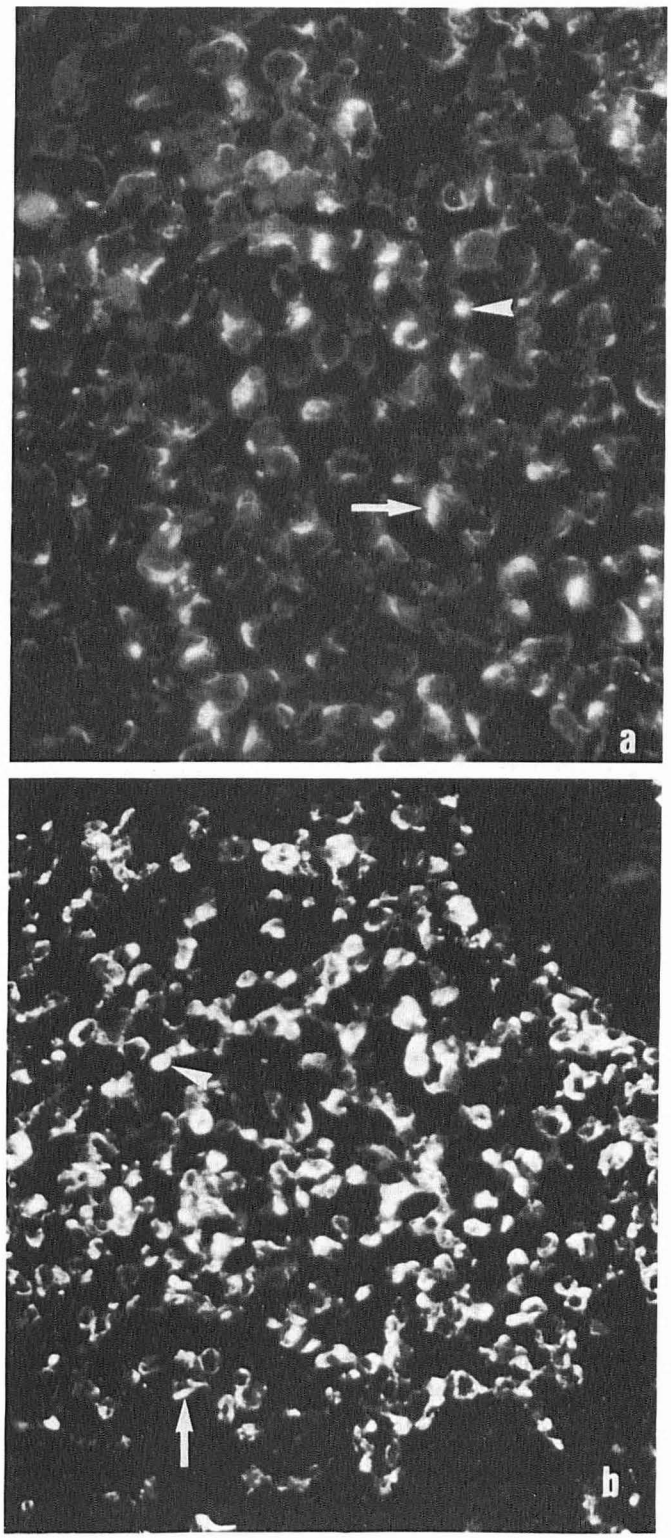

Figure 1. Single-labeling IIF. *a*, Case no. 1: about 75% of the NECA cells in this field show positive immunoreactivity with the antineurofilament antibodies (× 580). *b*, Case no. 3: Almost all NECA cells are cytokeratin positive (× 380). "Cord-like" (*arrow*) and "ball-like" (*arrowhead*) patterns.

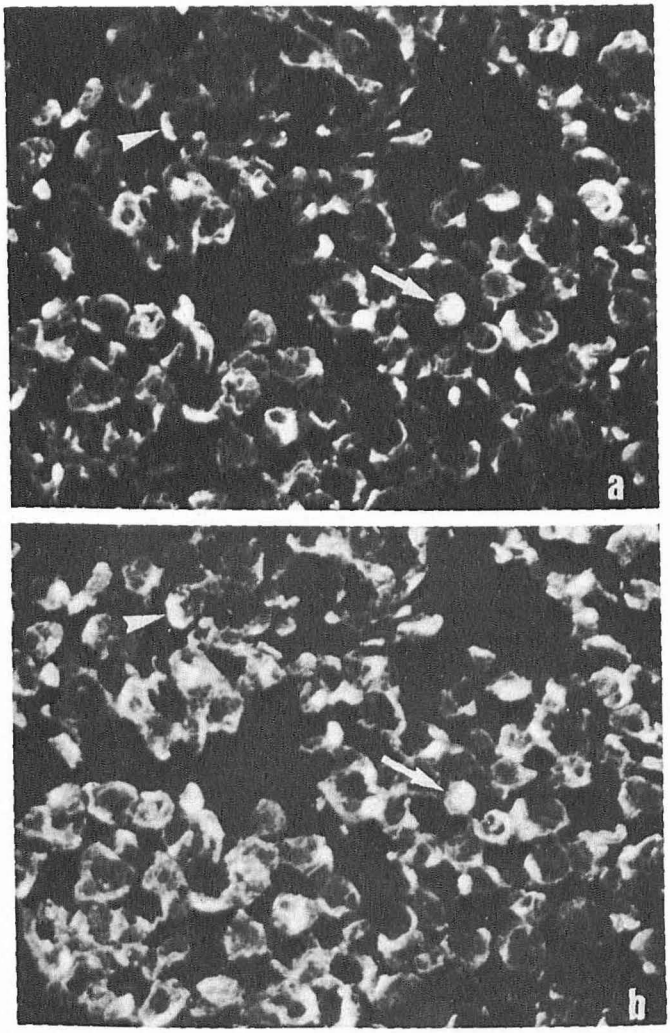

Figure 2. Double-labeling IIF with (*a*) antineurofilament and (*b*) antikeratin antibodies. The *arrow* and the *arrowhead* indicate cells stained with both antibodies (case no. 2) (× 580).

DISCUSSION

Our results demonstrate that NECA express cytokeratin polypeptides as well as neurofilament proteins, but not vimentin or desmin.§ Moreover, both intermediate filaments have been identified within the same cell by DIIF. Immunoreactivity of NECA cells with antineurofilament antibodies has already been reported, but the tumor cells stained negatively with antikeratin antibodies [25]. On the other hand, cytokeratin polypeptides were identified within NECA cells by another group [26]. This discrepancy may be, at least in part, explained by the nature of the antikeratin antisera used. Recently, both cytokeratin and neurofilament proteins were identified within NECA. However, both intermediate filaments were not shown to be within the same cell [27].

The coexpression of two distinct intermediate filament proteins (i.e., keratin and vimentin) by a unique cell is now well established and has been demonstrated in various in vivo (emybryonic and tumoral cells) and in vitro systems (for a review, see [28,29]), including cells of epithelial origin [30–34]. Both intermediate filaments can be demonstrated in the cells by electrophoretic analysis [32]. The coexisting subunits of the intermediate filaments can be components of distinct filaments in the cytoplasm, or form copolymers, i.e., being components of the same filaments [29]. However, up to this time, vimentin filaments appear to be the only class of intermediate filaments that has been demonstrated to be simultaneously expressed by the same cell in association with another class of intermediate filaments.

One objection that could be raised is that both antibodies used in this study recognized a common epitope of only one class of intermediate filaments (keratin or neurofilament) present in the NECA cells. Common antigenic determinants of all 5 classes of intermediate filaments have already been described [35]. However, the antibodies used in this study did not cross-react with each other in normal nonneoplastic conditions, and some NECA cells were keratin-positive and neurofilament-negative, while the reverse was true for other cells. Furthermore, the presence of both keratin polypeptides [36–38] and neurofilament proteins [39] has also been reported in the carcinoid tumor, another tumor of neuroendocrine origin. However, to our knowledge, there is as yet no report demonstrating the presence of both proteins in the same cells.

The origin of the NECA cell is still in question. Although it has been stated that these cells originate from the normal cutaneous MC [2–11], it is not likely that they are derived from the intraepidermal or intraappendageal MC, because the tumor only rarely involves the epidermis or surrounding appendageal epithelium [7,16,17]. Furthermore, the intraepithelial MC is an epidermal-derived cell, because it expresses a low-molecular-weight cytokeratin and not vimentin, desmin, glial fibrillar acidic protein, or neurofilament proteins [24,40–42].

An alternative explanation is that the NECA of the skin originates from another neuroendocrine cell which resides in the dermis (called dermal MC) [12–15], and is thought to be an in-transit MC coming from the neural crest [13–15]. Dual neuroendocrine cells have also been described in the appendix and bronchi. In these organs, extraepithelial neuroendocrine cells have been observed in close relationship with nerve endings within the normal submucosa [43–45], and have been implicated in the histogenesis of carcinoid tumors [43]. Although the intermediate filament proteins of the dermal MC remain to be established, it appears as though the NECA of the skin could arise from these cells.

§Margolis RJ, Merot Y, Dahl D, Bhan AK, Dickerson GR, Saurat JH, Mihm MC Jr: Simultaneous expression of cytokeratin and neurofilament-type intermediate filaments in neuroendocrine carcinoma of the skin. In preparation.

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